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(54) Title: NOVEL MU OPIOID RECEPTOR LIGANDS: AGONISTS AND ANTAGONISTS

(57) Abstract

The present invention provides novel opioid peptides. Disclosed are opioid peptides having the general structure Ac-Phe-Arg-Trp-Trp-Tyr-Xaa-NH_2 (SEQ ID No. 1); Ac-Arg-Trp-Ile-Gly-Trp-Xaa-NH_2 (SEQ ID No. 2); Trp-Trp-Pro-Lys/Arg-His_z-Xaa_z-NH_2 (SEQ ID No. 222); Tyr-Pro-Phe-Gly-Phe-Xaa-NH_2 (SEQ ID No. 5); (D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly_n-Xaa-NH_2 (SEQ ID No. 6); and (D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-Xaa-NH_2 (SEQ ID No. 7). Within each genus, Xaa is substituted by a specific amino acid. The invention also relates to an opioid peptide having general structure Tyr-A1-B2-C3-NH_2 (SEQ ID No. 8), wherein A is D-Nve or D-Nle, B is Gly, Phe, or Trp, and C is Trp or Nap. Also included within the invention are opioid compounds of the general structure Me_xH_yN-Tyr-Tyr-Phe_z-Pro_z-NH_2 (SEQ ID No. 221) which are peptides modified by permethylation, perallylation, perethylation, perbenzylation or pernaphthylation and which be further modified by reduction.

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NOVEL MU OPIOID RECEPTOR LIGANDS: AGONISTS AND ANTAGONISTS

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates generally to the field of peptide chemistry and, more specifically, to novel opioid peptides that can inhibit ligand binding to an opioid receptor.

BACKGROUND INFORMATION

There are at least three known subtypes of opioid receptors, mu (μ), delta (δ), and kappa (κ), to which morphine, the enkephalins, and the dynorphins, respectively, bind. The three receptor subtypes possess analgesic properties. However, the type of pain inhibited and the secondary functions vary with each receptor type. The μ receptor is generally regarded as the one associated with pain relief, respiratory depression, intestinal motility, antidiuresis, an immune response, and drug or other physical dependence. The δ receptor, on the other hand, is associated with thermal analgesia and, to a lesser extent, respiration and addiction. The κ receptor, though associated with dysphoric and psychometric effects, also has a lower potential for dependence as compared to the μ receptor. The κ receptor is potent in affecting analgesia in response to pain, including chemical stimuli. The κ receptor also induces diuresis and sedation. These differences in the opioid receptor functions encourage the search for drugs which produce analgesia without deleterious side effects.

The use of synthetic peptides has been instrumental in the delineation of these subtypes and in providing analogues that can be used for studying the

interactions of ligands specific to these receptor systems in both in vitro and in vivo systems. Certain opioid compounds are agonists (bind to the receptor and produce an effect) while others are antagonists (bind to the receptor but do not produce an effect). Most previously known agonists and antagonists of the opioid receptors are analogues of the enkephalins and related peptides, including the dynorphins, the dermenkephalins and the casomorphins. The compounds of the present invention have little to no sequence homology with any of these known opioid peptides.

Recent advances in methods for the preparation and screening of large numbers of individual peptides has led to the identification of numerous peptides useful in all areas of biomedical research, including research regarding the interaction of a ligand to the opiate receptor. Both receptor-specific agonists and antagonists are needed as pharmacological tools and as therapeutic agents. Even with these advances, however, basic research and drug discovery has been limited by the availability of the requisite large number of diverse opiate agonists and antagonists required to ascertain the relationship between a ligand for a particular opiate receptor subtype. Thus, a need exists for large numbers of individual compounds for use in biomedical research, including those for the study of opiate ligand-receptor interactions. As well there is a need for opioid peptides which have therapeutic value. This invention satisfies these needs and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides novel opioid peptides. These opioid peptides have the general structures Ac-Phe-Arg-Trp-Trp-Tyr-Xaa-NH_2 (SEQ ID NO. 1);

Ac-Arg-Trp-Ile-Gly-Trp-Xaa-NH_2 (SEQ ID NO. 2); Trp-Trp-Pro-Lys/Arg-His_z-Xaa_z-NH_2 (SEQ ID NO. 222); Tyr-Pro-Phe-Gly-Phe-Xaa-NH_2 (SEQ ID NO. 5); (D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly_n-Xaa-NH_2 (SEQ ID NO. 6); and (D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-Xaa-NH_2 (SEQ ID NO. 7). Within each above genus, Xaa is substituted by an amino acid. The invention also relates to an opioid peptide having the general structure Tyr-A1-B2-C3-NH_2 (SEQ ID NO. 214), wherein A1 is (D)Nve or (D)Nle, B2 is Gly, Phe, or Trp, and C3 is Trp or Nap. Also included within the invention are opioid compounds of the general structure Me_xH_yN-Tyr-Tyr-Phe_z-Pro_z-NH_2 (SEQ ID NO. 221) which are peptides modified by permethylation, perallylation, perethylation, perbenzylation or pernaphthylation and which can be further modified by reduction.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the two-dimensional chemical structures for the compounds of SEQ ID NOS. 162 through 167.

Figure 2 provides the two-dimensional chemical structures for the compounds identified by SEQ ID NOS. 168 to 173.

Figure 3 depicts the two-dimensional chemical structures for the compounds corresponding to SEQ ID NOS. 174 through 179.

Figure 4 shows the two-dimensional chemical structures for the compounds of SEQ ID. NOS. 180 to 185.

Figure 5 provides the two-dimensional chemical structures for the compounds corresponding to SEQ ID NOS. 186 through 191.

Figure 6 shows the two-dimensional chemical structures of the compounds corresponding to SEQ ID NOS. 192 through 197.

Figure 7 depicts the two-dimensional chemical structures for the compounds of SEQ ID NOS. 198 through 203.

Because the chemical structures provided in the Figures are two-dimensional, peptides of SEQ ID NOS. 208 to 213 which have at least one amino acid in the (D)-configuration correspond to the structure of SEQ ID NO. 162 of Figure 1.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel opioid peptides which are capable of inhibiting the binding of the μ-selective opioid peptide [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin. In one embodiment, the peptides have the general structure Ac-Phe-Arg-Trp-Trp-Tyr-Xaa-NH_2 (SEQ ID NO. 1), wherein Xaa is any one of the twenty naturally occurring amino acids.

In another embodiment, the novel peptides are those encompassed by the formula Ac-Arg-Trp-Ile-Gly-Trp-Xaa-NH_2 (SEQ ID NO. 2), where Xaa can be any one of the twenty naturally occurring amino acids.

In yet other embodiments of the present invention, the peptides have the structure Trp-Trp-Pro-Lys/Arg-His_z-Xaa_z-NH_2 (SEQ ID NO. 222) wherein Xaa can be any one of the twenty naturally occurring amino acids and z is 0 or 1, provided that if Xaa is present, His is present. More preferably, the peptides have the structure Trp-Trp-Pro-Lys-His-Xaa-NH_2 (SEQ ID NO. 3), where Xaa can be any one of the twenty naturally

occurring amino acids, or Trp-Trp-Pro-Lys/Arg-NH_2 (SEQ ID NO. 4). In the above formulae "Lys/Arg" means that either lysine or arginine may be substituted at that position.

Another embodiment of the invention provides peptides having the structure Tyr-Pro-Phe-Gly-Phe-Xaa-NH_2 (SEQ ID NO. 5), wherein Xaa can be any one of the twenty naturally occurring amino acids.

The invention also provides peptides falling within the structural formula (D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly_n-Xaa-NH_2 (SEQ ID NO. 6), wherein Xaa is Gly or the D-form of a naturally-occurring amino acid and n is 0 or 1. Peptides of this formula can be hexapeptides when Gly is absent (n is 0) and heptapeptides when Gly is present (n is 1).

Another embodiment also comprising D-amino acids are those peptides within the formula (D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-Xaa-NH_2 (SEQ ID NO. 7), wherein Xaa is Gly or the D-form of a naturally-occurring amino acid.

The invention also provides opioid peptides having the general structure Tyr-A1-B2-C3-NH_2 (SEQ ID NO. 214), wherein A1 is (D)Nve or (D)Nle, B2 is Gly, Phe, or Trp, and C3 is Trp or Nap.

Also included within the invention are opioid compounds which are peptide mimetics having the general structure Me_xH_yN-Tyr-Tyr-Phe_z-Pro_z-NH_2 (SEQ ID NO. 221), wherein x and y are 0, 1, or 2, with the proviso that x and y taken together is never greater than 2, and z is 0 or 1, provided that when Pro is present, Phe is present. Peptides falling within this formula are modified at the nitrogen atoms of the peptide backbone by permethylation,

perallylation, perethylation, perbenzylation and/or pernaphthylation. Such modification to the various nitrogens atoms within the backbone can be identical or different. Examples of where the modifications are different include peptides corresponding to SEQ ID NOs. 201 to 206 provided in Table 9 below. The peptides can be further modified by reduction as described further below. One or more of the amino acids can be in either the (L) or the (D)-configuration as exemplified, for example, in the compounds corresponding to SEQ ID NOs. 207 to 213.

The following standard abbreviations are used herein to identify amino acid residues.

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
NapthylAlaline	Nap	-
NorLeucine	Nle	-
NorValine	Nve	-
Phenylalanine	Phe	F
Proline	Pro	P

Serine	Ser	S
Threonine	Thr	T
Tetrahydroisoquinone-3-carboxylic acid	Tic	
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

The amino acids are indicated by these commonly known three and one letter codes as provided above and (D) designates an amino acid having the "D" configuration, as opposed to the naturally occurring L-amino acids. Unless otherwise indicated, where no specific configuration is indicated, one skilled in the art would understand the amino acid to be an (L)-amino acid. The alpha carbon atom of Gly is not asymmetric because it has two hydrogen atoms. Accordingly, Gly does not occur as a D or L isomer and is, therefore, not indicated to have either configuration.

As used herein, the phrase "any one of the twenty naturally occurring amino acids" means any one of the following: Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val. As used herein, the language "the D-form of a naturally-occurring amino acid" means the D-isomer of any one of these naturally-occurring amino acids, with the exception of Gly, which, as discussed above, does not occur as D or L isomers.

One skilled in the art would know that one or more amino acids within the exemplified peptides could be modified or substituted, as for example, by a conservative amino acid substitution of one or more of the specific amino acids shown in the exemplified peptides. A conservative amino acid substitution change can include, for example, the substitution of one acidic amino acid for another acidic amino acid, of one

hydrophobic amino acid for another hydrophobic amino acid or other conservative substitutions known in the art, including the use of non-naturally occurring amino acids, such as Nle for Leu or ornithine (Orn) or homoArginine (homoArg) for Arg.

In addition to the above types of modifications or substitutions, a mimic of one or more amino acids, otherwise known as a peptide mimetic or peptidomimetic, can also be used. As used herein, the term "mimic" means an amino acid or an amino acid analog that has the same or similar functional characteristic of an amino acid. Thus, for example, a (D)arginine analog can be a mimic of (D)arginine if the analog contains a side chain having a positive charge at physiological pH, as is characteristic of the guinidinium side chain reactive group of arginine. A peptide mimetic or peptidomimetic is an organic molecule that retains similar peptide chain pharmacophore groups as are present in the corresponding peptide.

The substitution of amino acids by non-naturally occurring amino acids and peptidomimetics as described above can enhance the overall activity or properties of an individual peptide based on the modifications to the side chain functionalities. For example, these types of alterations to the specifically exemplified peptides can enhance the peptide's stability to enzymatic breakdown and increase biological activity.

One skilled in the art, using the above formulae, can easily synthesize the peptides of this invention. Standard procedures for preparing synthetic peptides are well known in the art. The novel opioid peptides can be synthesized using the solid phase peptide synthesis (SPPS) method of Merrifield (J. Am. Chem. Soc., 85:2149 (1964), which is incorporated herein by reference) or modifications of SPPS, or the peptides can

be synthesized using standard solution methods well known in the art (see, for example, Bodanzsky, M., Principles of Peptide Synthesis 2nd revised ed. (Springer-Verlag, 1988 and 1993), which is incorporated herein by reference). Alternatively, simultaneous multiple peptide synthesis (SMPS) techniques well known in the art can be used. Peptides prepared by the method of Merrifield can be synthesized using an automated peptide synthesizer such as the Applied Biosystems 431A-01 Peptide Synthesizer (Mountain View, CA) or using the manual peptide synthesis technique described by Houghten, Proc. Natl. Acad. Sci., *USA* 82:5131 (1985), which is incorporated herein by reference.

Peptides can be synthesized using amino acids or amino acid analogs, the active groups of which are protected as necessary using, for example, a t-butyldicarbonate (t-BOC) group or a fluorenylmethoxy carbonyl (FMOC) group. Amino acids and amino acid analogs can be purchased commercially (Sigma Chemical Co.; Advanced Chemtec) or synthesized using methods known in the art. Peptides synthesized using the solid phase method can be attached to resins including 4-methylbenzhydrylamine (MBHA), 4-(oxymethyl)-phenylacetamido methyl and 4-(hydroxymethyl)phenoxy methylphenoxymethyl-copoly(styrene-1% divinylbenzene) (Wang resin), all of which are commercially available, or to p-nitrobenzophenone oxime polymer (oxime resin), which can be synthesized as described by De Grado and Kaiser, J. Org. Chem. 47:3258 (1982), which is incorporated herein by reference.

In the exemplified peptides, "Ac" indicates an acetyl group at the amino terminus and "NH_2" an amide group on the carboxy terminus. Peptides can be manipulated, for example, while still attached to a resin to obtain N-terminal modified compounds such as an

acetylated peptide or can be removed from the resin using hydrogen fluoride or an equivalent cleaving reagent and then modified. Compounds synthesized containing the C-terminal carboxy group (Wang resin) can be modified after cleavage from the resin or, in some cases, prior to solution phase synthesis. Methods for modifying the N-terminus or C-terminus such as methods for acetylation of the N-terminus or methods for amidation of the C-terminus are well known in the art.

Additional nomenclature used in the exemplified peptides includes, and means, the following: "Pm", permethylated; "Pa", perallylated; "Pe", perethylated; "Pb", perbenzylated; "Pn", pernaphthylated. The methylation, allylation, ethylation, benzylation, and naphthylation in the respective peptides is at each of the nitrogen atoms in the peptide backbone as shown in Figures 1 through 7. Modification of the amide backbone by permethylation and the like yields peptidomimetics with diverse physio-chemical properties different from the peptides from which they were obtained, such as enhanced stability to enzymatic breakdown and increased biological activity. Various methods for permethylation and the like have been described, including Ostresh et al., Proc.Natl. Acad. Sci. USA, 91:11138 (1994), Hakomori, S., J. Biochem., 55:205 (1964), Challis and Challis, The Chemistry of Amides, pp.731 (1970), all of which are incorporated herein by reference.

The reduction of the peptide amides is another means for the chemical transformation of peptides which adds stability and can enhance activity. In the exemplified peptides, the use of "red" means that the carbonyls of the amide peptide backbone are reduced to amines, as shown, for instance, in Figures 1 through 7 and more specifically, for example, in Figure 1, number 162. A number of reagents are available and well known

for the reduction of amides to amines such as those disclosed in Wann et al., JOC, 46:257 (1981) and Raucher et al., Tett.Let., 21:14061 (1980), both of which are incorporated herein by reference. Diborane has the advantage that trimethylborate, the only by-product in the reaction workup, is volatile and is therefore readily removed by evaporation. The use of excess diborane in refluxing tetrahydrofuran permits simple aliphatic and aromatic amides to be rapidly, and often quantitatively be reduced into their corresponding amines.

A newly synthesized peptide can be purified using a method such as reverse phase high performance liquid chromatography (RP-HPLC) or other methods of separation based on the size or charge of the peptide. Furthermore, the purified peptide can be characterized using these and other well known methods such as amino acid analysis and mass spectrometry.

After manufacture, the peptides can be assayed for receptor binding activity using the radioreceptor assay (Examples I and II) or other assays outlined below, including the adenylyl cyclase assay (Example III), or the guinea-pig ileum assay (Example IV) or the mouse vas deferens assay (Example IV). In addition, the warm water mouse tail-flick assay is an in vivo animal model useful for testing peptides of the present invention. The tail-flick assay is described, for example, in Dooley et al., Science, 266:2019-2022 (1994) and Jiang et al., J. Pharmacol. Exp. Ther., 262:526 (1992), both of which are incorporated herein by reference.

Because the peptides of the present invention bind to the μ receptor, they can be used in in vitro assays to study the opiate receptor subtypes. For example, in a sample receptor of unknown type or origin, the peptides, after being labeled with a detectable

marker such as a radioisotope, can be contacted with the receptor sample under conditions which specifically favor binding to a particular receptor subtype. Unbound receptor and peptide can be removed, for example, by washing with a saline solution, and bound receptor can then be detected using methods well known to those skilled in the art. Therefore, the peptides of the present invention are useful in vitro for the diagnosis of relevant opioid receptor subtypes, and in particular the μ type, in brain and other tissue samples.

In addition to their utility in in vitro screening methods, the peptides are also useful in vivo. For example, the opioid peptides can be used in vivo diagnostically to localize opioid receptor subtypes. The peptides are also useful as drugs to treat pathologies associated with other compounds which interact with the opioid receptor system. Therefore, the peptides of the present invention can be used in medicaments for treating pathologies associated with the μ receptor and as analgesics. It can be envisioned that these peptides can be used for therapeutic purposes to block the peripheral effects of a centrally acting pain killer. For instance, morphine is a centrally acting pain killer. Morphine, however, has a number of deleterious effects in the periphery which are not required for the desired analgesic effects, such as constipation and pruritus (itching). While it is known that the many peptides do not readily cross the blood-brain barrier and, therefore, elicit no central effect, the subject peptides can have value in blocking the periphery effects of morphine, such as constipation and pruritus.

The novel peptides claimed can be incorporated into pharmaceutical compositions. Pharmaceutically acceptable carriers are well known in the art and include aqueous solutions such as physiologically buffered saline

or other buffers or solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters. A pharmaceutically acceptable carrier can contain physiologically acceptable compounds that act, for example, to stabilize the opioid peptide or increase the absorption of the peptide. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration and on the particular physio-chemical characteristics of the specific opioid peptide.

Methods of administering a pharmaceutical are well known in the art and include but are not limited to administration orally, intravenously, intramuscularly or intraperitoneal. Administration can be effected continuously or intermittently and will vary with the subject and is dependent on the type of treatment and potency of the peptide used.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

Identification Of Mu Selective Opioid Peptides By A Radioreceptor Assay

This example describes the identification of individual peptides, either contained within a synthetic combinatorial library mixture or prepared separately, as inhibitors of the μ-selective opioid peptide [^{3}H]-[D-Ala^2, MePhe^4, Gly-Ol^5 enkephalin ([^{3}H]-DAMGO). Individual

peptides were identified as capable of inhibiting [^{3}H]-DAMGO by a radioreceptor assay.

Synthetic combinatorial libraries (SCLs) made up of mixtures of tens of millions of different peptides can be used to rapidly identify individual, active compounds. Since the libraries are in solution (i.e., not attached to a bead, pin, phage, glass, etc.) they can be screened in virtually any assay system.

With the exception of the permethylated and reduced peptides, all opioid peptides were initially prepared and contained within SCLs. Some SCLs were composed entirely of L-amino acids, one of which contained an N-terminal acetyl moiety, while other SCLs were composed primarily of D-amino acids as detailed by the peptide structures provided below. The libraries were used in conjunction with an iterative selection process to identify individual peptides capable of inhibiting tritiated DAMGO in the radioreceptor assay.

As detailed below, the libraries were screened at a single concentration (0.08 mg/ml) in a radioreceptor assay using rat brain homogenates and [^{3}H]-DAMGO as radioligand. Ic_{50} values were determined for mixtures in the library which significantly inhibited the binding of [^{3}H]-DAMGO.

Radioreceptor Assays Selective For The Mu Receptor

Rat and guinea pig brains, frozen in liquid nitrogen, were obtained from Harlan Bioproducts for Science (Indianapolis, IN). Frozen brains were thawed, the cerebella removed and the remaining tissue weighed. Each brain was individually homogenized in 40 ml Tris-HCl buffer (50 mM, pH 7.4, 4°C) and centrifuged (39000 x g) (Model J2-HC; Beckman Instruments, Fullerton, CA) for 10

min at 4°C. The pellets were resuspended in fresh Tris-HCl buffer and incubated at 37°C for 40 min. Following incubation, the suspensions were centrifuged as above, the resulting pellets resuspended in 100 volumes of Tris buffer and the suspensions combined. Membrane suspensions were prepared and used in the same day. Protein content of the crude homogenates ranged from 0.15-0.2 mg/ml as determined using the method described by Bradford (Bradford, Anal. Biochem. 72:248-254 (1976), which is incorporated herein by reference).

Binding assays were carried out in polypropylene tubes. Each tube contained 0.5 ml of membrane suspension, 3 nM of the μ-selective opioid peptide [^{3}H]-DAMGO (specific activity 36 Ci/mmol), 0.08 mg/ml peptide mixture and Tris-HCl buffer in a total volume of 0.65 ml. Assay tubes were incubated for 60 min at 25°C. The reaction was terminated by filtration through GF-B filters (Wallac, Inc., Gaithersburg, MD). The filters were subsequently washed with 6 ml Tris-HCl buffer at 4°C. Bound radioactivity was counted on a Beta-plate Liquid Scintillation Counter (Life Technologies, Gaithersburg, MD) and expressed in counts per minute (cpm). Inter- and intra-assay variation standard curves were determined by incubation of [^{3}H]-DAMGO in the presence of 0.13-3900 nM of unlabeled DAMGO. Competitive inhibition assays were performed as above using serial dilutions of the peptide mixtures. IC_{50} values were then calculated using the software GRAPHPAD (ISI, San Diego, CA). IC_{50} values of less than 1000 nM are indicative of highly active opioid peptides which bind to the μ receptor, with particularly active compounds having IC_{50} values of 100 nM or less and the most active compounds with values of less than 10 nM.

Opioid peptides having the general structure Ac-Phe-Arg-Trp-Trp-Tyr-Xaa-NH_2 (SEQ ID NO. 1), wherein Xaa is any one of the twenty naturally-occurring amino acids were identified. IC_{50} values of these peptides are provided in Table 1.

TABLE 1

Ac-Phe-Arg-Trp-Trp-Tyr-Xaa-NH_2
(SEQ ID NO. 1)

SEQ ID NO.	PEPTIDE	IC_{50} (nM)
8	Ac-Phe-Arg-Trp-Trp-Tyr-Met-NH_2	33
9	Ac-Phe-Arg-Trp-Trp-Tyr-Leu-NH_2	35
10	Ac-Phe-Arg-Trp-Trp-Tyr-Ser-NH_2	39
11	Ac-Phe-Arg-Trp-Trp-Tyr-Ala-NH_2	50
12	Ac-Phe-Arg-Trp-Trp-Tyr-Ile-NH_2	64
13	Ac-Phe-Arg-Trp-Trp-Tyr-Thr-NH_2	73
14	Ac-Phe-Arg-Trp-Trp-Tyr-Val-NH_2	77
15	Ac-Phe-Arg-Trp-Trp-Tyr-Gly-NH_2	78
16	Ac-Phe-Arg-Trp-Trp-Tyr-Phe-NH_2	170
17	Ac-Phe-Arg-Trp-Trp-Tyr-Arg-NH_2	191
18	Ac-Phe-Arg-Trp-Trp-Tyr-Lys-NH_2	221
19	Ac-Phe-Arg-Trp-Trp-Tyr-Gln-NH_2	234
20	Ac-Phe-Arg-Trp-Trp-Tyr-Tyr-NH_2	257
21	Ac-Phe-Arg-Trp-Trp-Tyr-His-NH_2	272
22	Ac-Phe-Arg-Trp-Trp-Tyr-Trp-NH_2	326
23	Ac-Phe-Arg-Trp-Trp-Tyr-Cys-NH_2	400
24	Ac-Phe-Arg-Trp-Trp-Tyr-Asn-NH_2	486
25	Ac-Phe-Arg-Trp-Trp-Tyr-Pro-NH_2	668
26	Ac-Phe-Arg-Trp-Trp-Tyr-Asp-NH_2	769
27	Ac-Phe-Arg-Trp-Trp-Tyr-Glu-NH_2	1480

Also identified from screening of the acetylated SCL were peptides of the general formula Ac-Arg-Trp-Ile-Gly-Trp-Xaa-NH_2 (SEQ ID NO. 2), wherein Xaa is any one of the twenty naturally-occurring amino acids. Table 2 provides the respective IC_{50} values for each of the twenty peptides within this formula.

TABLE 2

Ac-Arg-Trp-Ile-Gly-Trp-Xaa-NH_2
(SEQ ID NO.2)

SEQ ID NO.	PEPTIDE	IC_{50} (nM)
28	Ac-Arg-Trp-Ile-Gly-Trp-Arg-NH_2	5
29	Ac-Arg-Trp-Ile-Gly-Trp-Lys-NH_2	35
30	Ac-Arg-Trp-Ile-Gly-Trp-Thr-NH_2	51
31	Ac-Arg-Trp-Ile-Gly-Trp-Met-NH_2	175
32	Ac-Arg-Trp-Ile-Gly-Trp-Ile-NH_2	224
33	Ac-Arg-Trp-Ile-Gly-Trp-Ala-NH_2	444
34	Ac-Arg-Trp-Ile-Gly-Trp-Phe-NH_2	539
35	Ac-Arg-Trp-Ile-Gly-Trp-Ser-NH_2	574
36	Ac-Arg-Trp-Ile-Gly-Trp-Leu-NH_2	620
37	Ac-Arg-Trp-Ile-Gly-Trp-Cys-NH_2	651
38	Ac-Arg-Trp-Ile-Gly-Trp-Trp-NH_2	703
39	Ac-Arg-Trp-Ile-Gly-Trp-Tyr-NH_2	976
40	Ac-Arg-Trp-Ile-Gly-Trp-Val-NH_2	1070
41	Ac-Arg-Trp-Ile-Gly-Trp-Gln-NH_2	1390
42	Ac-Arg-Trp-Ile-Gly-Trp-His-NH_2	2010
43	Ac-Arg-Trp-Ile-Gly-Trp-Gly-NH_2	2050
44	Ac-Arg-Trp-Ile-Gly-Trp-Pro-NH_2	3090
45	Ac-Arg-Trp-Ile-Gly-Trp-Asn-NH_2	3120
46	Ac-Arg-Trp-Ile-Gly-Trp-Glu-NH_2	6150
47	Ac-Arg-Trp-Ile-Gly-Trp-Asp-NH_2	6480

Screening of a non-acetylated, all L-amino acid library revealed that peptides of the formula Trp-Trp-Pro-Lys-His-Xaa-NH_2 (SEQ ID NO. 3) inhibited the μ-selective radioligand. Again, Xaa can be any one of

twenty naturally-occurring amino acids as shown in Table 3, along with their respective IC_{50} values. In addition two shorter analogues, Trp-Trp-Pro-Lys-NH_2 (SEQ ID NO. 68) and Trp-Trp-Pro-Arg-NH_2 (SEQ ID NO. 69) were identified, the IC_{50} values of which are also provided in Table 3.

TABLE 3

Trp-Trp-Pro-Lys-His-Xaa-NH_2 (SEQ ID NO. 3) and Trp-Trp-Pro-Lys/Arg-NH_2 (SEQ ID NO. 4)

SEQ ID NO.	PEPTIDE	IC_{50} (nM)
48	Trp-Trp-Pro-Lys-His-Gly-NH_2	9
49	Trp-Trp-Pro-Lys-His-Asn-NH_2	11
50	Trp-Trp-Pro-Lys-His-Lys-NH_2	13
51	Trp-Trp-Pro-Lys-His-His-NH_2	14
52	Trp-Trp-Pro-Lys-His-Ser-NH_2	15
53	Trp-Trp-Pro-Lys-His-Arg-NH_2	17
54	Trp-Trp-Pro-Lys-His-Ala-NH_2	17
55	Trp-Trp-Pro-Lys-His-Gln-NH_2	18
56	Trp-Trp-Pro-Lys-His-Ile-NH_2	31
57	Trp-Trp-Pro-Lys-His-Pro-NH_2	32
58	Trp-Trp-Pro-Lys-His-Thr-NH_2	33
59	Trp-Trp-Pro-Lys-His-Cys-NH_2	33
60	Trp-Trp-Pro-Lys-His-Val-NH_2	34
61	Trp-Trp-Pro-Lys-His-Met-NH_2	42
62	Trp-Trp-Pro-Lys-His-Phe-NH_2	50
63	Trp-Trp-Pro-Lys-His-Glu-NH_2	55
64	Trp-Trp-Pro-Lys-His-Tyr-NH_2	55
65	Trp-Trp-Pro-Lys-His-Leu-NH_2	79
66	Trp-Trp-Pro-Lys-His-Asp-NH_2	81
67	Trp-Trp-Pro-Lys-His-Trp-NH_2	187
68	Trp-Trp-Pro-Lys-NH_2	17
69	Trp-Trp-Pro-Arg-NH_2	10

Also identified from a non-acetylated, L-amino acid SCL were peptides of the formula Tyr-Pro-Phe-Gly-Phe-Xaa-NH_2 (SEQ ID NO. 5). The IC_{50} values for each of the peptides of this formula are provided in Table 4.

TABLE 4

Tyr-Pro-Phe-Gly-Phe-Xaa-NH_2
(SEQ ID NO. 5)

SEQ ID NO.	PEPTIDE	IC_{50} (nM)
70	Tyr-Pro-Phe-Gly-Phe-Arg-NH_2	13
71	Tyr-Pro-Phe-Gly-Phe-Gly-NH_2	18
72	Tyr-Pro-Phe-Gly-Phe-Lys-NH_2	19
73	Tyr-Pro-Phe-Gly-Phe-Ser-NH_2	24
74	Tyr-Pro-Phe-Gly-Phe-Ala-NH_2	27
75	Tyr-Pro-Phe-Gly-Phe-Asn-NH_2	28
76	Tyr-Pro-Phe-Gly-Phe-Pro-NH_2	31
77	Tyr-Pro-Phe-Gly-Phe-Gln-NH_2	33
78	Tyr-Pro-Phe-Gly-Phe-Met-NH_2	33
79	Tyr-Pro-Phe-Gly-Phe-His-NH_2	38
80	Tyr-Pro-Phe-Gly-Phe-Tyr-NH_2	41
81	Tyr-Pro-Phe-Gly-Phe-Ile-NH_2	43
82	Tyr-Pro-Phe-Gly-Phe-Thr-NH_2	45
83	Tyr-Pro-Phe-Gly-Phe-Leu-NH_2	48
84	Tyr-Pro-Phe-Gly-Phe-Val-NH_2	50
85	Tyr-Pro-Phe-Gly-Phe-Phe-NH_2	61
86	Tyr-Pro-Phe-Gly-Phe-Asp-NH_2	75
87	Tyr-Pro-Phe-Gly-Phe-Cys-NH_2	81
88	Tyr-Pro-Phe-Gly-Phe-Trp-NH_2	87
89	Tyr-Pro-Phe-Gly-Phe-Glu-NH_2	119

Peptides of the general formula (D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly_n-Xaa-NH_2 (SEQ ID NO. 6) were also inhibitors of [^{3}H]-DAMGO binding. IC_{50} for the hexapeptides (n=0) of the formula (D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Xaa-NH_2 (SEQ ID NO. 215), and for the heptapeptides (n=1) of the formula (D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-Xaa-NH_2 (SEQ ID NO. 216), wherein Xaa is Gly or the D-form of a naturally-occurring amino acid, are provided in Tables 5 and 6, respectively.

TABLE 5

(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Xaa-NH_2
(SEQ ID NO. 215)

SEQ ID NO.	PEPTIDE	IC_{50} (nM)
90	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-NH_2	10
91	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Ala-NH_2	12
92	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)His-NH_2	16
93	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Val-NH_2	17
94	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Tyr-NH_2	18
95	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Ser-NH_2	20
96	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Ile-NH_2	20
97	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Arg-NH_2	22
98	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Met-NH_2	26
99	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Asn-NH_2	29
100	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Gln-NH_2	30
101	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Pro-NH_2	32
102	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Thr-NH_2	36
103	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Trp-NH_2	58
104	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Asp-NH_2	60
105	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Glu-NH_2	64
106	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Cys-NH_2	79
107	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Lys-NH_2	118
108	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Phe-NH_2	153
109	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-(D)Leu-NH_2	2541

TABLE 6

(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-Xaa-NH_2
(SEQ ID NO. 216)

SEQ ID NO.	PEPTIDE	IC_{50} (nM)
110	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Phe-NH_2	1
111	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Lys-NH_2	4
112	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Met-NH_2	9
113	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Pro-NH_2	10
114	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Ile-NH_2	10
115	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Arg-NH_2	10
116	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)His-NH_2	10
117	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Tyr-NH_2	13
118	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Thr-NH_2	14
119	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-Gly-NH_2	14
120	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Ser-NH_2	14
121	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Trp-NH_2	15
122	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Leu-NH_2	18
123	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Ala-NH_2	18
124	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Asn-NH_2	18
125	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Val-NH_2	18
126	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Gln-NH_2	20
127	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Glu-NH_2	78
128	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Cys-NH_2	94
129	(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly-(D)Asp-NH_2	97

Table 7 provides the IC_{50} values of the opioid peptides identified as inhibitors which are within the general formula (D)Ile-(D)Met-(D)Thr-(D)-Trp-Gly-Xaa-NH_2 (SEQ ID NO. 7), wherein Xaa is Gly or the D-form of a naturally occurring amino acid.

TABLE 7

(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-Xaa-NH_2
(SEQ ID NO. 7)

SEQ ID NO.	PEPTIDE	IC_{50} (nM)
130	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Pro-NH_2	15
131	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Tyr-NH_2	16
132	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Phe-NH_2	27
133	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Met-NH_2	35
134	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Trp-NH_2	37
135	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Ile-NH_2	38
136	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Ala-NH_2	45
137	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Leu-NH_2	49
138	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-Gly-NH_2	58
139	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Cys-NH_2	58
140	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Val-NH_2	71
141	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)His-NH_2	86
142	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Ser-NH_2	87
143	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Arg-NH_2	97
144	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Lys-NH_2	107
145	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Asn-NH_2	109
146	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Thr-NH_2	130
147	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Gln-NH_2	139
148	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Asp-NH_2	1709
149	(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-(D)Glu-NH_2	1959

Identified from a positional scanning synthetic combinatorial library (PS-SCL) are the peptides of Table 8 which have the generic formula Tyr-A1-B2-C3-NH2 (SEQ. ID NO.8), wherein A1 is (D)Nve or (D)Nle, B2 is Gly, Phe, or Trp, and C3 is Trp or Nap. IC_{50} values for each of the peptides in the μ selective radioreceptor assay are provided below.

TABLE 8

Tyr-A1-B2-C3-NH_2
(SEQ ID NO. 214)

SEQ ID NO.	PEPTIDE	IC_{50} (nM)
150	Tyr-(D)Nve-Gly-Nap-NH_2	0.2
151	Tyr-(D)Nle-Gly-Nap-NH_2	1
152	Tyr-(D)Nve-Gly-Trp-NH_2	3
153	Tyr-(D)Nle-Gly-Trp-NH_2	5
154	Tyr-(D)Nve-Phe-Trp-NH_2	5
155	Tyr-(D)Nve-Trp-Nap-NH_2	5
156	Tyr-(D)Nve-Trp-Trp-NH_2	5
157	Tyr-(D)Nve-Phe-Nap-NH_2	7
158	Tyr-(D)Nle-Phe-Trp-NH_2	15
159	Tyr-(D)Nle-Trp-Trp-NH_2	17
160	Tyr-(D)Nle-Phe-Nap-NH_2	30
161	Tyr-(D)Nle-Trp-Nap-NH_2	32

Synthesis of Tyr-Tyr, Tyr-Tyr-Phe, and Tyr-Tyr-Phe-Pro (SEQ ID NO. 217) Related Compounds

Specific compounds related to the di-tri-and tetra-peptides Tyr-Tyr, Tyr-Tyr-Phe, and Tyr-Tyr-Phe-Pro (SEQ ID NO. 217), respectively, and encompassed by the formula Me_xH_yN-Tyr-Tyr-Phe_z-Pro_z-NH_2 (SEQ ID NO. 221) were

synthesized and their receptor binding studied. The protected tetrapeptide resin YYFP-MBHA (SEQ ID NO. 217) and the tripeptide resin YYF-MBHA were permethylated, perethylated, perallylated, perbenzylated and pernaphthylated in their trityl protected form to obtain, after trityl deprotection, the free N-terminal amino groups. Furthermore these peptide resins were also modified by reductive alkylation prior to permethylation and perallylation.

Reductive alkylation was performed using the dimethoxydityl-protected peptide resins to obtain the monomethylated N-terminal amine and the free amine to obtain the dimethylated N-terminal amine. One series of these twenty peptides (including one non alkylated peptide sequence as a control) was cleaved from the resin after peralkylation - one series was reduced after peralkylation. Synthetic details are given below for the representative example Pm and red {MeHN-YYF-NH_2}.

The following materials were used. Amino acid derivatives were purchased from Bachem California (Torrance, CA). p-Methylbenzhydrylamine resin (MBHA), 1% DVB, 100-200 mesh, 0.9 meq./g substitution, was received from Peninsula Laboratories, INC (Belmont, CA). Anhydrous THF, anhydrous DMSO and lithium tert. butoxide as a 1 M solution in THF were purchased from Aldrich (Milwaukee, WI). Other solvents were obtained from Fisher (Fair Lawn, NJ).

The peptide starting material protected peptide resin, of Trt-Tyr (2,6-di-Cl-Bzl)-Tyr(2,6-di-Cl-Bzl)-Phe-MBHA was synthesized using standard Boc chemistry and simultaneous multiple peptide synthesis starting with 100 mg MBHA resin (0.09 mmol amino groups) contained in a polypropylene mesh packet.

Triphenylmethyl chloride (Trityl chloride, TrtCl) was coupled as follows. The Tyr(2,6-di-Cl-Bzl)-Tyr(2,6-di-Cl-Bzl)-Phe-MBHA resin (0.09 mmol primary amino groups) was washed with DCM (1 x 1 min x 5ml), 5% DIEA/DCM (3 x 2 min x 5 ml) and DCM (2 x 1 min x 5 ml). 5.84 ml DMF/DCM (9:1) and 0.455 ml DIEA (2.61 mmol) were added to the peptide-resin, followed by addition of 125.45 mg TrtCl (0.45 mmol). The reaction mixture was shaken for 6 hr. The peptide resin was washed with DCM (1 x 1 min x 5 ml), 5% DIEA/DCM (1 x 1 min x 5 ml) and /DCM (1 x 1 min x 5 ml). The coupling was repeated for 2 hr using 9 ml DCM as the solvent and the same amounts of DIEA and TrtCl as used for the first coupling. The peptide-resin was washed with DMF (2 x 1 min x 5 ml), 5% DIEA/DCM (1 x 2 min x 5 ml), DCM (3 x 1 min x 5 ml) and MeOH (1 x 1 min x 5 ml). A small sample of peptide-resin was tested for the completeness of the coupling using the bromophenol blue test (Krchnak et al., Collect. Czech. Chem. Commun. 53: 2542-2548 (1988)).

The resin-bound protected peptide was peralkylated as follows. All manipulations were performed under nitrogen atmosphere and strictly anhydrous conditions. The peptide-resin was dried overnight at 50 mTorr. 10.8 ml 0.5 M Lithium tert. butoxide in THF (5.4 mmol) was added to the Trt-Tyr (2,6-di-Cl-Bzl)-Tyr(2,6-die-Cl-Bzl)-Phe-MBHA resin packet (0.09 mmol peptide, 0.27 mmol amide groups) and shaken at room temperature for 15 min. The excess base solution was removed using a positive nitrogen pressure syphon. 10.8 ml DMSO and 1.008 ml iodomethane (16.2 mmol) were added. The reaction mixture was shaken at room temperature for 2 hr. The alkylation solution was removed by positive nitrogen pressure syphon transfer and the entire procedure repeated twice.

The resin packet was washed with DMF (3 x 1 min x 5 ml), IPA (2 x 1 min x 5 ml), DCM (3 x 1 min x 5 ml) and MeOH (1 x 1 min x 5 ml).

The trityl group was deprotected as follows. The peptide-resin was washed with DCM (1 x 1 min x t ml) and then treated with 2% TFA in DCM (1 x 2 min x 5 ml and 1 x 30 min x 5 ml), followed by the 1 min. washing steps DCM (1 x 5 ml), IPA (2 x 5 ml) and DCM (2 x 5 ml).

Reductive methylation of 4, 4'-dimethoxydityl (Dod) protected unmodified or permethylated peptides can be done according to the procedures of Kaljuste and Unden, Int. J. Peptide Protein Res. 42:118-124 (1993). Coupling of 4,4'-dimethoxydityl chloride (DodCl) was performed as follows. The Tyr(2,6-di-Cl-Bzl)-(NMe)-Tyr(2,6-di-Cl-Bzl)-(NMe)-Phe-(NMe)-MBHA resin packet (0.09 mmol primary amine) was washed with DCM (1 x 1 min x 5 ml), 5% DIEA/DCM (3 x 2 min x 5 ml) and DCM (2 x 1 min x t ml). 22.5 ml DCM and 0.455 ml DIEA (2.61 mmol) were added to the resin packet, followed by addition of 118.17 mg of DodCl (0.45 mmol). The mixture was shaken for 90 min. The resin packet was washed with DMF (2 x 1 min x 5 ml), 5% DIEA/DCM (1 x 2 min x 5 ml) and DCM (3 x 1 min x 5 ml). A sample of the resin was tested for the completeness of the coupling using the bromophenol blue test.

Formaldehyde solution was prepared by mixing 10 ml formaldehyde (37% wt solution in water), 90 ml DMF and 30 g of anhydrous magnesium sulfate. The solution was shaken for 24 hr, centrifuged, and then the supernatant used for the reductive methylation.

For reductive methylation, 10.8 ml formaldehyde solution were added to Dod-Tyr(2,6-di-Cl-Bzl)-(NMe)-Tyr(2,6-di-Cl-Bzl)-(NMe)-Phe-(NMe)MBHA resin packet (0.09

mmol peptide) and shaken for 5 min. The solution was poured off. Additional 10.8 ml formaldehyde solution with 0.108 ml acetic acid were added and shaken. After 5 min, 108 mg of sodium cyanoborohydride was added and the mixture shaken for 1 hr. The resin packet was washed with DMF (2 x 1 min x 5 ml), IPA (2 x 1 min x 5 ml), DCM (3 x 1 min x 5 ml) and MeOH (1 x 1 min x 5 ml).

The Dod protecting group was removed by washing the resin packet with DCM (1 x 1 min x 5 ml), followed by treatment with 55% TRA in DCM (1 x 5 min x 5 ml and 1 x 30 min x 5 ml), followed by the 1 min washing steps DCM (1 x 5 ml, IPA (2 x 5 ml) and DCM (2 x 1 min).

Reduction was as follows. Into a 50 ml glass tube (teflon-lined cap) were added the Dod-(NMe)-Tyr(2,6-di-Cl-Bzl)-(NMe)-Tyr(2,6-di-Cl-Bzl)-(Nme)-Phe-(NMe)MBHA resin packet and 310 mg boric acid (5.014 mmol). Under nitrogen atmosphere, 0.5 ml trimethylborate (0.0042 mmol) were added, followed by the addition of 15 ml of 1 M borane-tetrahydrofurane complex (15 mmol). Following cessation of hydrogen evolution, the tube was sealed and placed in a controlled temperature oven at 65° for 100 hr. The tubes were then removed, cooled to room temperature and 2 ml methanol were added to quench excess reducing agent. The resin packet was washed with THF (1 x 1 min x 10 ml) and MeOH (4 x 1 min x 10 ml). After drying the resin packet, it was covered with 15 ml piperidine and heated at 65° C for 18 hr. The resin packet was washed with DMF (2 x 1 min x 5 ml), DCM (2 x 1 min x 5 ml), MeOH (1 x 1 min x 5 ml), DMF (2 x 1 min x 5 ml), DCM (2 x 1 min x 5 ml) and MeOH (1 x 1 min x 5 ml).

Peptide was released from the solid support by the following. The resin packet was cleaved with hydrogen fluoride (5 ml with 0.35 ml anisole added as scavenger) using a multiple vessel cleavage apparatus for

9 hr at 0°C (Houghten et al., Int. J. Pept. Protein Res., 27:673-678 (1986)). The resulting polyamine was extracted using 50% aqueous acetonitrile (3 x 5 ml) following sonication. The solution was lyophilized, taken up twice more in 50% aqueous acetonitrile and lyophilized yielding the crude product 2.

Table 9 provides the IC_{50} values of the opioid compounds synthesized which are within the general formula Me_xH_yN-Tyr-Tyr-Phe_z-Pro_z-NH_2 (SEQ ID NO. 221).

TABLE 9

SEQ ID NO.	PEPTIDE	IC_{50} (nM)
162	Pm and red {Me_2N-Tyr-Tyr-Phe-NH_2}	1
163	Pm and red {MeHN-Tyr-Tyr-Phe-NH_2}	88
164	Pm and red {NH_2-Tyr-Tyr-Phe-NH_2}	14
165	Pa and red {Me_2N-Tyr-Tyr-Phe-NH_2}	181
166	Pa and red {MeHN-Tyr-Tyr-Phe-NH_2}	178
167	Pa and red {NH_2-Tyr-Tyr-Phe-NH_2}	244
168	Pe and red {NH_2-Tyr-Tyr-Phe-NH_2}	409
169	Pb and red {NH_2-Tyr-Tyr-Phe-NH_2}	4906
170	Pn and red {NH_2-Tyr-Tyr-Phe-NH_2}	7466
171	Pm {Me_2N-Tyr-Tyr-Phe-NH_2}	5857
172	Pm {MeHN-Tyr-Tyr-Phe-NH_2}	6244
173	Pm {NH_2-Tyr-Tyr-Phe-NH_2}	288
174	Pa {Me_2N-Tyr-Tyr-Phe-NH_2}	10953
175	Pa {MeHN-Tyr-Tyr-Phe-NH_2}	27064
176	Pa {NH_2-Tyr-Tyr-Phe-NH_2}	21923
177	Pe {NH_2-Tyr-Tyr-Phe-NH_2}	11025
178	Pb {NH_2-Tyr-Tyr-Phe-NH_2}	26937
179	Pn {NH_2-Tyr-Tyr-Phe-NH_2}	24014
180	Pm and red {Me_2N-Tyr-Tyr-Phe-Pro-NH_2}	8

TABLE 9

SEQ ID NO.	PEPTIDE	IC_{50} (nM)
181	Pm and red {MeNH-Tyr-Tyr-Phe-Pro-NH_2}	2
182	Pm and red {NH_2-Tyr-Tyr-Phe-Pro-NH_2}	13
183	Pa and red {Me_2N-Tyr-Tyr-Phe-Pro-NH_2}	69
184	Pa and red {MeHN-Tyr-Tyr-Phe-Pro-NH_2}	51
185	Pa and red {NH_2-Tyr-Tyr-Phe-Pro-NH_2}	66
186	Pe and red {NH_2-Tyr-Tyr-Phe-Pro-NH_2}	45
187	Pb and red {NH_2-Tyr-Tyr-Phe-Pro-NH_2}	4464
188	Pn and red {NH_2-Tyr-Tyr-Phe-Pro-NH_2}	18589
189	Pm {Me_2N-Tyr-Tyr-Phe-Pro-NH_2}	5857
190	Pm {MeHN-Tyr-Tyr-Phe-Pro-NH_2}	6244
191	Pm {NH_2-Tyr-Tyr-Phe-Pro-NH_2}	2014
192	Pa {Me_2N-Tyr-Tyr-Phe-Pro-NH_2}	21524
193	Pa {MeHN-Tyr-Tyr-Phe-Pro-NH_2}	10047
194	Pa {NH_2-Tyr-Tyr-Phe-Pro-NH_2}	21923
195	Pe {NH_2-Tyr-Tyr-Phe-Pro-NH_2}	11025
196	Pb {NH_2-Tyr-Tyr-Phe-Pro-NH_2}	26937
197	Pn {NH_2-Tyr-Tyr-Phe-Pro-NH_2}	24014
198	Pm and red {Me_2N-Tyr-Tyr-NH_2}	194
199	Pm and red {MeHN-Tyr-Tyr-NH_2}	80
200	Pm and red {H_2N-Tyr-Tyr-NH_2}	4143
201	red {Me_2N-Tyr-(NMe)-Tyr-(NHBzl)-NH_2}	34
202	red {MeHN-Tyr-(NMe)-Tyr-(NHBzl)-NH_2}	2
203	red {H_2N-Tyr-(NMe)-Tyr-(NHBzl)-NH_2}	8
204	red {Me_2N-Tyr-(NHBzl)-Tyr-(NHMe)-NH_2}	1143
205	red {MeHN-Tyr-(NHBzl)-Tyr-(NHMe)-NH_2}	1047
206	red {H_2N-Tyr-(NHBzl)-Tyr-(NHMe)-NH_2}	739
207	Pm and red {Me_2N-Tyr-Tyr-(D)Phe-NH_2}	38
208	Pm and red {Me_2N-Tyr-(D)Tyr-Phe-NH_2}	352

TABLE 9

SEQ ID NO.	PEPTIDE	IC_{50} (nM)
209	Pm and red {Me_2N-(D)Tyr-Tyr-Phe-NH_2}	687
210	Pm and red {Me_2N-(D)Tyr-(D)Tyr-(D)Phe-NH_2}	866
211	Pm and red {Me_2N-(D)Tyr-(D)Tyr-Phe-NH_2}	1554
212	Pm and red {Me_2N-Tyr-(D)Tyr-(D)Phe-NH_2}	908
213	Pm and red {Me_2N-(D)Tyr-Tyr-(D)Phe-NH_2}	993

EXAMPLE II

Mu Receptor Specificity Of Opioid Peptides

This example demonstrates the specificity of the novel opioid peptides for the μ receptors as compared to the δ and κ opiate receptors.

Individual peptides were synthesized and the activity of these peptides in the radioreceptor assay selective for the μ receptors from Example I were compared to radioreceptor assays selective for the δ and κ receptors as detailed below.

Individual peptides were synthesized using simultaneous multiple peptide synthesis (SMPS) and their identities confirmed by mass spectral analysis on a MALDI instrument from Kratos Analytical (Ramsey, NJ). Peptides were purified by reversed-phase high performance liquid chromatography using a Waters Milliprep 300 preparative HPLC (Milford, MA) modified with a Gilson Model 232 preparative autosampler and Foxy fraction collector (Gilson Medical Electronics, Middleton, WI). Pure fractions (determined using analytical HPLC) were pooled and lyophilized.

Radioreceptor assays selective for δ receptors were performed using rat brain homogenates as above and [^{3}H]-naltrindole (0.5 nM, specific activity 34.7 Ci/mmol) as radioligand in Tris buffer containing 100 μM phenylmethylsulfonyl fluoride (PMSF), 5 mM $MgCl_2$ and 1 mg/ml bovine serum albumin (BSA), pH 7.4. Samples were incubated for 2.5 hr. Standard curves were prepared using 0.10-3200 nM [^{3}H]-D-Pen_2, Pen_5]-enkephalin ([^{3}H]-DPDPE).

Assays selective for κ receptors were carried out using [^{3}H]-U69,593 (3 nM, specific activity 62 Ci/mmol) as the radioligand and tissue homogenates prepared from guinea pig brains (cortex and cerebellum) using Tris buffer containing 100 μM PMSF, 5 mM $MgCl_2$ and 1 mg/ml BSA, pH 7.4. Sample tubes were incubated for 2.5 hr. Standard curves were prepared using 0.05-6300 nM naloxone.

Tritiated ligands, [^{3}H]-DAMGO, [^{3}H]-DPDPE and [^{3}H]-[D-Ser^2, Leu^5, Thr^6]enkephalin ([^{3}H]-DSLET), Abuse (NIDA) repository, as prepared by Multiple Peptide Systems (San Diego, CA), [^{3}H]-U69,593 from Amersham (Arlington Heights, IL) and [^{3}H]-naltrindole from DuPont NEN Research Products (Los Angeles, CA). The average standard deviation for IC_{50} values was ±20%.

TABLE 10

PEPTIDE SELECTIVITY

PEPTIDE	SEQ ID NO.	μ IC_{50} (nM)	δ IC_{50} (nM)	κ IC_{50} (nM)	δ/μ	κ/μ
YPFGFR-NH_2	70	13	26,500	5,050	2,040	388
YPFGFG-NH_2	71	18	18,300	38,600	1,010	2,150
YPFGFK-NH_2	72	19	69,800	10,400	3,670	549

TABLE 10

PEPTIDE SELECTIVITY

PEPTIDE	SEQ ID NO.	μ IC_{50} (nM)	δ IC_{50} (nM)	κ IC_{50} (nM)	δ/μ	κ/μ
YPFGFS-NH_2	73	24	38,800	69,100	1,620	2,880
WWPKHG-NH_2	48	9	7,660	81,100	851	9,010
WWPKHN-NH_2	49	11	42,600	70,700	3,880	6,430
WWPKHK-NH_2	50	13	90,600	33,200	6,970	2,560
WWPKHH-NH_2	51	14	53,900	66,900	3,850	4,780
WWPK-NH_2	68	17	35,000	80,000	2,060	4,700
WWPR-NH_2	69	10	17,400	20,600	1,740	2,060
Ac-RWIGWR-NH_2	28	5	1,670	502	330	100
Ac-RWIGWK-NH_2	29	35	5,620	4,240	160	121
Ac-RWIGWT-NH_2	30	51	6,120	21,200	120	415
Ac-RWIGWM-NH_2	31	175	2,900	20,800	17	119
Ac-FRWWYM-NH_2	8	33	940	28,700	28	870
Ac-FRWWYL-NH_2	9	35	1,540	21,000	44	600
Ac-FRWWYI-NH_2	12	64	193	99,400	3	1,550
Ac-FRWWYG-NH_2	15	78	303	8,720	4	112
Y-(D)Nve-G-Nap-NH_2	150	0.2	11	203	55	1015
Y-(D)Nle-G-Nap-NH_2	151	1	24	1178	24	1178
Y(D)NveGW-NH_2	152	3	199	1701	66	567
Y(D)NleGW-NH_2	153	5	220	2250	44	450
Y(D)NveFW-NH_2	154	5	127	228	25	46
Y-(D)Nve-W-Nap-NH_2	155	5	98	214	20	43
Y(D)NveWW-NH_2	156	5	282	752	56	150
Y-(D)Nve-F-Nap-NH_2	157	7	652	1221	93	174
Y(D)NleFW-NH_2	158	15	303	816	20	54
Y(D)NleWW-NH_2	159	17	517	2045	30	120

TABLE 10

PEPTIDE SELECTIVITY

PEPTIDE	SEQ ID NO.	μ IC_{50} (nM)	δ IC_{50} (nM)	κ IC_{50} (nM)	δ/μ	κ/μ
Y-(D)Nle-F-Nap-NH_2	160	30	1055	1859	35	62
Y-(D)Nle-W-Nap-NH_2	161	32	449	1226	14	38
DAMGO	--	3	758	2,530	253	844
DALDA	--	14	271,800	6,800	19,400	485
YGGFL-OH	218	65	41	16,700	0.7	257

The above comparative data provided in Table 10 demonstrates the selectivity of the novel opioid peptides for the μ receptors over the δ and κ opiate receptors.

EXAMPLE III

Mu Receptor Agonist Or Antagonist Activity By Adenylyl Cyclase Assay

Certain opioid compounds are agonists (bind to the receptor and produce an effect) while others are antagonists (bind to the receptor but do not produce an effect). This example uses the adenylyl cyclase assay to demonstrate that certain of the novel opioid peptides are agonists of the μ receptor while others are antagonists of the μ receptor.

An adenylyl cyclase assay using human SH-SY5Y neuroblastoma cell line membranes was used to rapidly determine the opioid agonists or antagonist properties of individual peptides. Opioid agonist inhibit adenylyl cyclase activity, resulting in reduced levels of cyclic AMP.

Adenylyl Cyclase Assay

The human SH-SY5Y neuroblastoma cell line (Biedler et al., Cancer Research, 38:3751-3757 (1978), which is incorporated herein by reference) was cultured in RPMI 1640 medium buffered with 12.5 mM HEPES, pH 7.2, and which contained 300 μg/ml L-glutamine, 100 μg/ml penicillin, 100 μg/ml streptomycin, 50 μM 2-mercaptoethanol, 60 μM 2-ethanolamine and 10% iron-supplemented bovine calf serum in 5% CO_2 at 37°C. Cells were cultured in medium containing 10 μM retinoic acid for six days before harvesting in order to differentiate the cells as previously described (Ya and Sadfe, J. Pharmacol. Exp. Ther., 245:350-355 (1988), which is incorporated herein by reference).

Cell membranes were prepared for use in the adenylyl cyclase assays as previously described (Law et al., Mol. Pharmacol., 23:26-35 (1983), which is herein incorporated by reference). After the initial centrifugation at 200 x g for 15 min at 4°C, the cells were resuspended in sucrose buffer (0.32 M sucrose, 40 mM HEPES, 2 mM EGTA, pH 7.6). Cells were centrifuged again at 200 x g, then homogenized in sucrose buffer with five strokes of a Dounce Homogenizer (Wheaton Instruments, Millville, NJ). Membranes were centrifuged at 22000 x g for 20 min at 4°C, then resuspended in sucrose buffer. The protein concentration was determined by the method of Bradford (Bradford, *supra*), using BSA as the standard. SH-SY5Y membranes at a protein concentration of 1-4 mg/ml were stored at -80°C.

SH-SY5Y membranes were incubated in a final volume of 100 μl of 40 mM HEPES, pH 7.7, containing 15 units of creatine phosphokinase, 20 mM phosphocreatine, 1mM 1,10-o-phenanthroline, 60 μM isobutylmethylxanthine

(IBMX), 50 μM ATP, 50 μM GTP, 3 mM $MgCl_2$ and 100 mM NaCl. Each of the agonists and antagonists tested were included at final concentrations of 100 μM and 500 μM, respectively. The reaction was initiated by the addition of 36 μg of membrane protein. After 15 min at 30°C, the reaction was stopped by the addition of 100 μl of cold 4.5% perchloric acid. The samples were neutralized by the addition 40 μl of cold 30% potassium bicarbonate. Finally, the membranes were centrifuged at 12000 x g for 4 min at 4°C in a microcentrifuge. The amount of cyclic AMP present in 100 μl of the supernatant, equivalent to the cyclic AMP produced by 15 μg of membrane protein, was determined using a cyclic AMP kit (Diagnostic Products, Los Angeles, CA). This procedure, which uses a cyclic AMP binding protein in a competitive protein binding assay, is based on the method of Tovey et al. (Clin. Chim. Acts, 56:221-234), which is incorporated herein by reference), and was used with the following modification: [^{3}H]-cyclic AMP (specific activity 31.4 Ci/mmol), obtained from Amersham (Arlington Heights, IL), was used instead of the [^{3}H]-cyclic AMP included in the assay kit. [^{3}H]-cyclic AMP, 0.9 μCi, was added into 6 ml of H_2O, and 100 μl of the diluted [^{3}H]-cyclic AMP was added to the assay tubes. The final supernatants were counted in 10 ml of Ecolite (+) scintillation fluid (ICN Pharmaceuticals, Covina, CA).

A reduction of cyclic AMP levels to less than 70% the basal cyclic AMP levels was regarded as being indicative of an opioid agonist effect, provided that the inhibition of cyclic AMP was blocked by the opioid antagonist naloxone. Table 11 provides the results of the adenylyl cyclase assay and indicates which of the tested peptides are μ receptor agonists and which are μ receptor antagonists.

TABLE 11

ADENYLYL CYCLASE ASSAY FOR MU RECEPTOR AGONIST OR ANTAGONIST ACTIVITY

	SEQ ID NO.	% Basal Control		
AGONISTS			+ Naloxone	+ICI 174,864
YPFGFR-NH_2	70	53 ± 3	77 ± 8	28 ± 3
WWPKHN-NH_2	49	43 ± 10	92 ± 3	26 ± 3
WWPKHG-NH_2	48	35 ± 4	80 ± 0.6	32 ± 2
WWPK-NH_s	68	47 ± 8	101 ± 2	36 ± 5
WWPR-NH_2	69	35 ± 3	91 ± 3	34 ± 3
Ac-FRWWYM-NH_2	8	46 ± 1	70 ± 2	30 ± 0.3
DAMGO (100 μM)	--	34 ± 6	77 ± 8	28 ± 3
ANTAGONISTS			+ Naloxone	+ICI 174,864
Ac-RWIGWR-NH_2	28	105 ± 12		
DAMGO (1 μM)	--	61 ± 5	101 ± 4	95 ± 3

EXAMPLE IV

Mu Receptor Agonist Or Antagonist Activity By Guinea-Pig Ileum And Mouse Vas Deferens Assays

This example demonstrates by guinea-pig ileum and mouse vas deferens assays that certain of the novel opioid peptides are agonists of the receptor while others are antagonists.

Guinea-Pig Ileum Assay

The guinea-pig ileum (GPI) bioassay was carried out to determine whether a peptide is an opioid agonist (binds to receptor and initiates intracellular signal) or an opioid antagonist (binds to receptor but does not initiate an intracellular signal). Such information

cannot be determined from the above-described radioreceptor binding assay.

The GPI assay (Kosterlitz et al., Br. J. Pharam. 39:398-418 (1970), which is incorporated herein by reference), is one of the most widely used assays for the determination of opioid activities in vitro. The assay is based on the ability of opioid agonists to inhibit electrically stimulated contraction in tissue. GPI is not a "clean" assay insofar as the ileum preparation contains both μ and κ receptors. However, μ receptor mediated effects in the GPI can be distinguished from κ receptor mediated effects by determining K_e values for naloxone as the antagonist (μ effects: K_e ~ 1-3 nM); κ effects: K_e ~ 20-30 nM) or by using specific μ or κ antagonists.

The guinea pigs were killed by decapitation. The ileum was removed and longitudinal muscle-myenteric plexus preparations were placed in Krebs solution (NaCl 118 mM, KCL 4.75 mM, NaH_2PO_4 1 mM, $NaHCO_3$ 25 mM, $MgSO_4$ 1.2 mM, glucose 11 mM and $CaCl_2$ 2.5 mM). The solution was gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. The tissue was suspended under a final tension of 1 g in a 10 ml organ bath and stabilized for 1 hr. Electrical stimulation via a straight platinum electrode was applied, 0.4 ms pulses of supramaximal voltage, delivered at a rate of 0.1 Hz. Isometric contractions were measured via strain gauge force transducers and recorded on stripchart recorders.

Mouse Vas Deferens Assay

The mouse vas deferens (MVD) bioassay was also used to determine whether a peptide is an opioid agonist or an opiod antagonist. Again, such information is not available from the radioreceptor binding assay results.

The MVD assay (Henderson et al., Br. J. Pharma. 12:119-127 (1957), which is incorporated herein by reference) is another one of the most widely used assays for the determination of opioid activities in vitro. This assay, like the GPI assay, is based on the ability of opioid agonists to inhibit electrically stimulated contractions in tissue. The MVD assay is also not "clean" in that, in addition to the predominant δ receptor, the μ and κ receptors are also present. However, the use of a specific δ antagonist, such as H-Tyr-Tic-Phe-Phe-NH_2 (SEQ ID NO. 223) (TIPP) permits an assessment as to whether or not an agonist effect observed in the MVD assay is indeed δ receptor mediated.

Mouse vasa deferentia were dissected and placed in Krebs solution. The responses of the longitudinal muscle were recorded under a basal tension of 0.5 g and excited by pulses of 1 millisecond duration.

In all bioassays, a dose-response curve with a reference compound (e.g., [D-Ala^2, Leu^5]enkephelinamide or U50,488) is used in each preparation. This is important, since IC_{50} values of a given compound can vary considerably from one preparation to another. Potencies of new compounds to be tested are determined relative to that of the reference compound, and their IC_{50} values are normalized based on an average IC_{50} value which has been obtained for the reference compound by performance of many determinations with a large number of preparations. In cases where peptides might be susceptible to enzymatic degradation, potencies are determined in the presence of a mixture of peptidase inhibitors (l-leucyl-leucine, 2 mM; Bestatin, 10-30 μM; thiorphan, 0.3 μM; captopril, 10 μM), as recommended by McKnight et al., Eur. J. Pharm. 86:339-402 (1983). K_e values for naloxone or other antagonists are determined from the ratio of IC_{50} values (DR) obtained with the peptide under investigation in the

presence and absence of a fixed concentration of the antagonist, using the formula $K_e = a/(DR-1)$ ("a" = fixed concentration). A log dose-response curve is obtained with [Leu^5]enkephalin for each ileum and vas deferens preparation and the IC_{50} value is determined. K_e values for antagonists are determined from the ratio of IC_{50} values obtained with [Leu^5]enkephalin in the presence and absence of a fixed antagonist concentration.

A log dose-response curve was obtained with [Leu^5]enkephalin for each ileum and vas preparation and the IC_{50} value determined. K_e values for antagonists were determined from the ratio of IC_{50} values obtained with [Leu^5]enkephalin in the presence and absence of a fixed antagonist concentration of naloxone (Kosterlitz and Watt, Br. J. Pharamcol., 33:266-276 (1968), which is incorporated herein by reference).

Table 12 provides the results of two peptides, WWPKHN-NH_2 (SEQ ID NO. 49) and WWPKHK-NH_2 (SEQ ID NO. 50) tested in the GPI and MVP assays. These peptides were shown to be agonists in both assays. Their potencies were similar to that of leucine-enkephalin (YGGFL-OH) (SEQ ID NO. 218) in the GPI assay. The agonist effects of WWPKHN-NH_2 (SEQ ID NO. 49) and WWPKHK-NH_2 (SEQ ID NO. 50) in the GPI assay were antagonized by naloxone with K_e values of 1.27 ± 0.16 nM and 2.97 ± 0.43 nM, respectively, indicating that they were μ receptor-mediated. While the IC_{50} values in the MVD assay were much lower than those of the GPI assay, the MVD assay as discussed above, is predominantly responsive to compounds acting on the δ receptors. Activity in the MVD assay was not antagonized by TIPP, a highly δ-selective antagonist. It was, however, antagonized by naloxone, which preferentially antagonizes μ receptor-mediated effects and therefore, the activity observed in the MVD assay is mediated by the μ receptors presence in the tissue. The

activity observed in these assays shows that the subject peptides have potential analgesic activity in vivo.

TABLE 12

AGONIST ACTIVITIES IN THE GUINEA PIG ILEUM (GPI) AND MOUSE VAS DEFERENS (MVP) ASSAYS

PEPTIDE	SEQ ID NO.	GPI IC_{50}(nM)	MVD IC_{50}(nM)	GPI/MVD ratio
WWPKHN-NH_2	49	220 ± 10	31 ± 10	7.07
WWPKHK-NH_2	20	270 ± 19	45 ± 7	6.07
YGGFL-OH	218	246 ± 4	11 ± 1	21.6

Additional individual peptides shown to have agonist or antagonist activity either by the adenylyl cyclase assay or the GPI assay are provided in Table 13.

TABLE 13

MU RECEPTOR AGONIST OR ANTAGONIST ACTIVITY

PEPTIDE	SEQ ID NO.	AGONIST OR ANTAGONIST
Pm and red {Me_2N-Tyr-Tyr-Phe-NH_2}	162	Antagonist
Ac-RFMWMK-NH_2	219	Antagonist
(D)I-(D)M-(D)S-(D)W-(D)W-G-NH_2	220	Agonist
(D)I-(D)M-(D)T-(D)W-G-(D)P-NH_2	15	Agonist

Although the invention has been described with reference to the examples provided above, it should be understood that various modifications can be made by those skilled in the art without departing from the invention. Accordingly, the invention is set out in the following claims.

WE CLAIM:

1. A peptide having the structure:

Ac-Phe-Arg-Trp-Trp-Tyr-Xaa-NH_2

(SEQ ID NO. 1), wherein Xaa is a naturally-occurring amino acid.

2. A peptide having the structure:

Ac-Arg-Trp-Ile-Gly-Trp-Xaa-NH_2

(SEQ ID NO. 2), wherein Xaa is a naturally-occurring amino acid.

3. A peptide having the structure:

Trp-Trp-Pro-Lys/Arg-His_z-Xaa_z-NH_2 (SEQ ID NO. 222),

wherein z is 0 or 1; and
wherein Xaa is a naturally-occurring amino acid, provided that when Xaa is present, His is present.

4. The peptide of claim 3, having the structure:

Trp-Trp-Pro-Lys-His-Xaa-NH_2 (SEQ ID NO. 3),

wherein Xaa is a naturally-occurring amino acid.

5. The peptide of claim 3, having the structure:

Trp-Trp-Pro-Lys/Arg-NH_2 (SEQ ID NO. 4).

6. A peptide having the structure:

Tyr-Pro-Phe-Gly-Phe-Xaa-NH_2

(SEQ ID NO. 5), wherein Xaa is a naturally-occurring amino acid.

7. A peptide having the structure:

(D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly_n-Xaa-NH_2

(SEQ ID NO. 6), wherein n is 0 or 1 and wherein Xaa is Gly or the D-form of a naturally-occurring amino acid.

8. A peptide having the structure:

(D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-Xaa-NH_2

(SEQ ID NO. 7), wherein Xaa is Gly or the D-form of a naturally-occurring amino acid.

9. A peptide having the structure:

Tyr-A1-B2-C3-NH_2 (SEQ ID NO. 214);

wherein A1 is (D)Nve or (D)Nle;
B2 is Gly, Phe, or Trp; and
C3 is Trp or Nap.

10. A peptide having the structure:

Me_xH_yN-Tyr-Tyr-Phe_z-Pro_z-NH_2 (SEQ ID NO. 221), wherein x is 0, 1, or 2 and y is 0, 1, or 2, provided that x and y together is never greater than 2; z is 0 or 1, provided that when Pro is present, Phe is present; and provided that the nitrogen atoms in the peptide backbone between the respective amino acids are

modified, wherein the modification is selected from the group consisting of permethylated, perallylated, perethylated, perbenzylated, and pernaphthylated.

11. The peptide of claim 10, wherein all of the carbonyls of the amide peptide backbone are reduced.

12. The peptide of claim 10, wherein any one or more of the amino acids are in the (D)-configuration.

13. The peptide of claim 10, wherein the modification for all the nitrogen atoms in the peptide backbone are identical modifications.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09321

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 38/08
US CL :514/17
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/17

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, CAS Online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	US 5,455,230 A (P. W. SCHILLER) 03 October 1995 (03.10.95).	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search
22 AUGUST 1996

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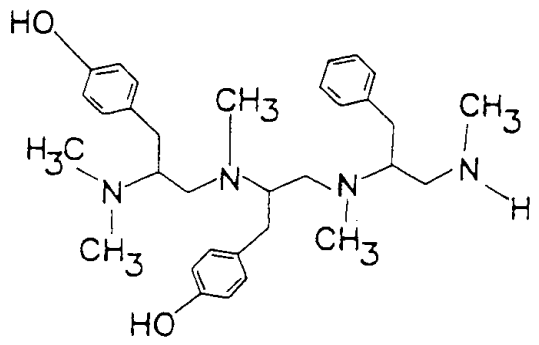

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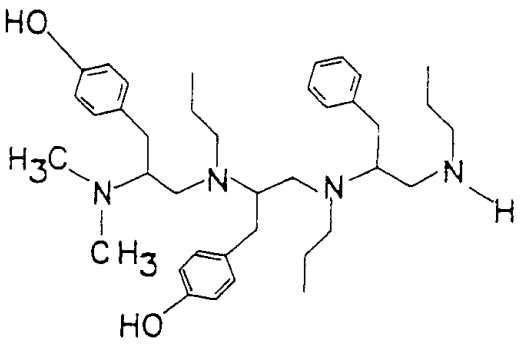

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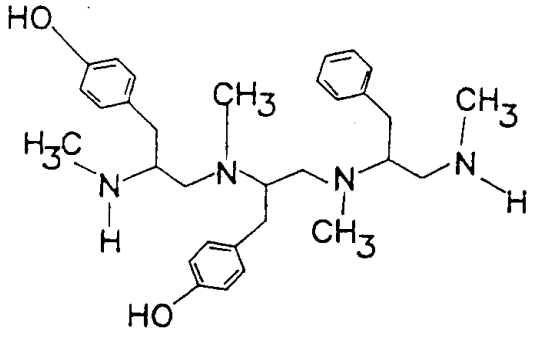

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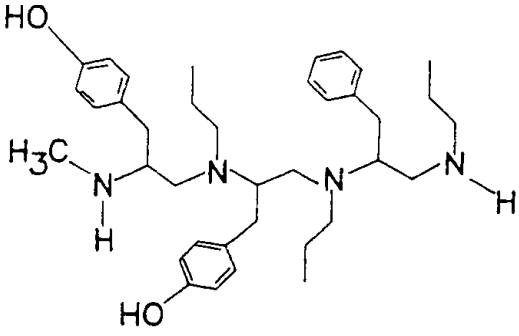

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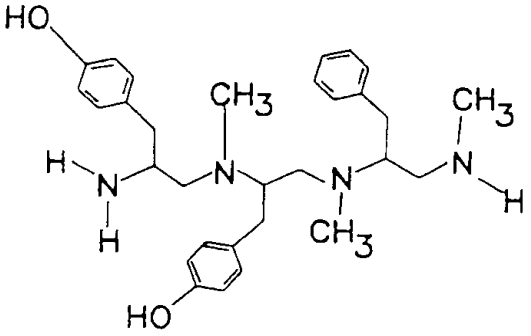

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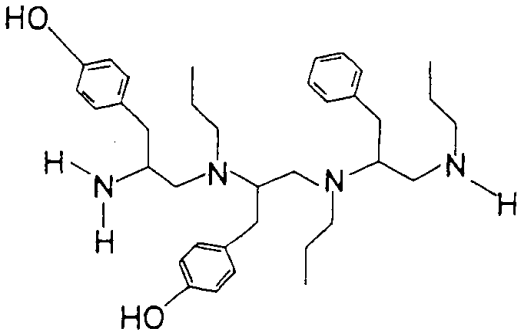

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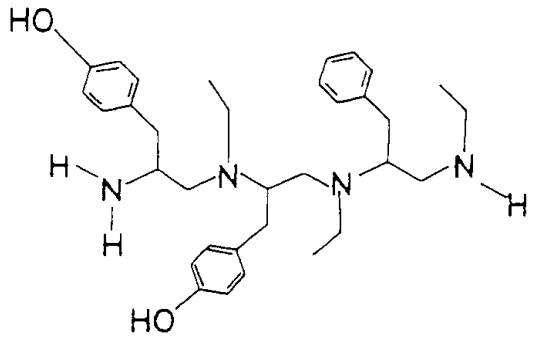
FIG. 1



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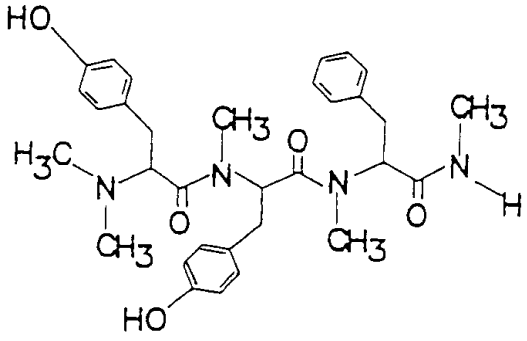

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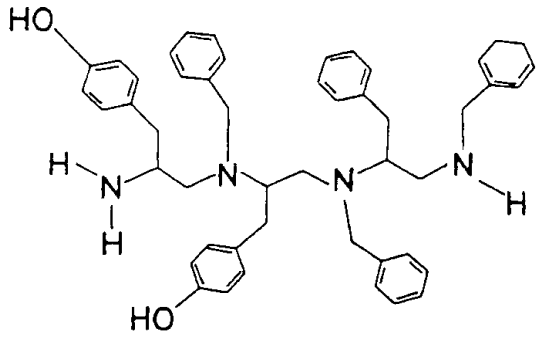

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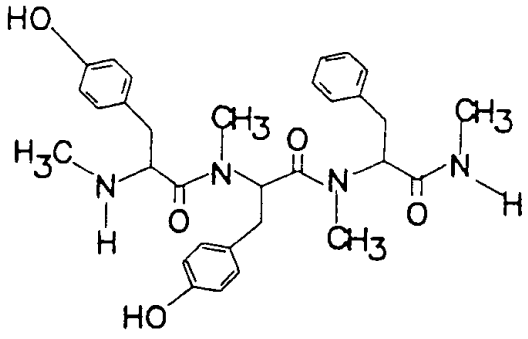

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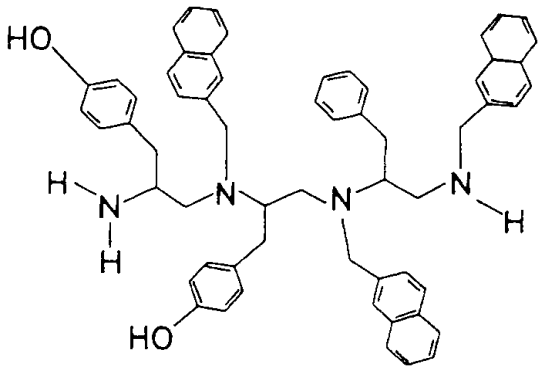

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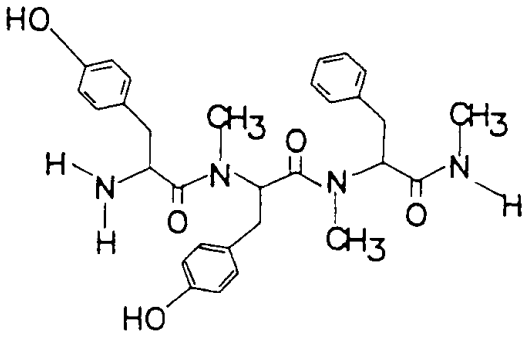
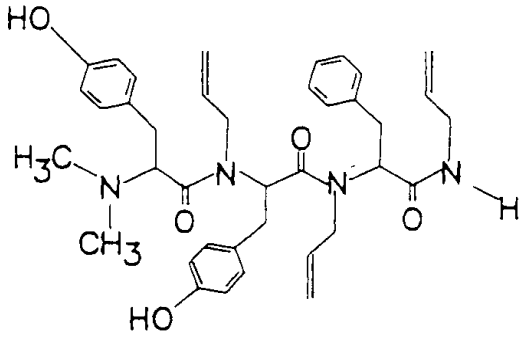


173

FIG. 2



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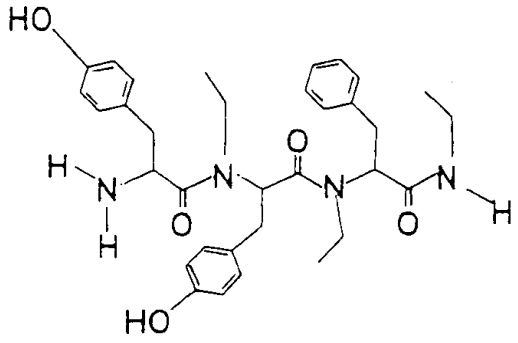

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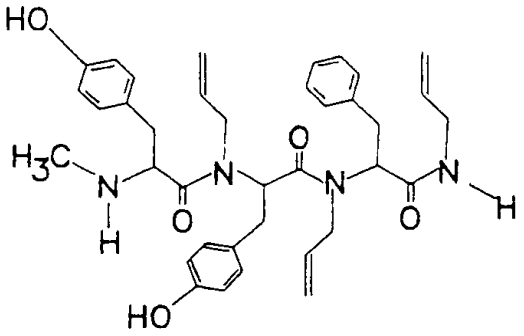

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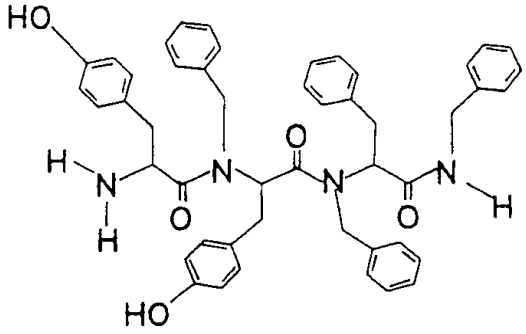

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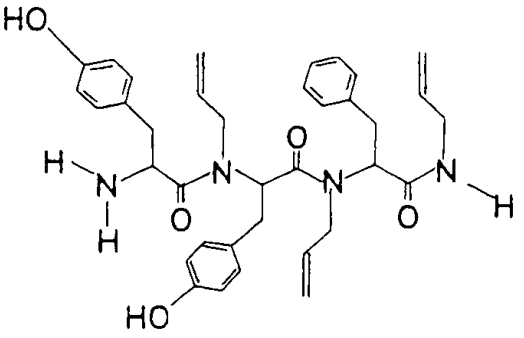

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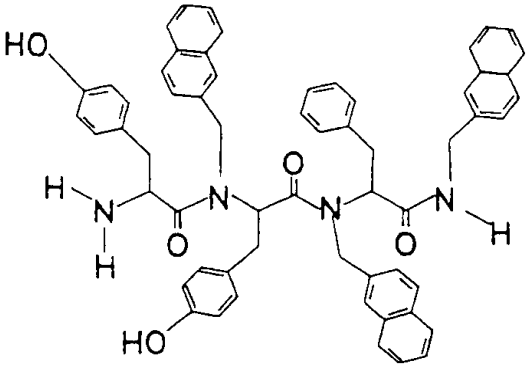
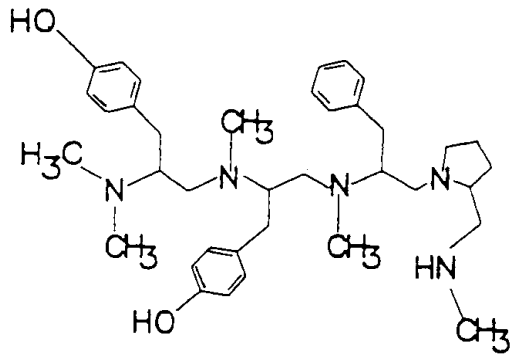


179

FIG. 3



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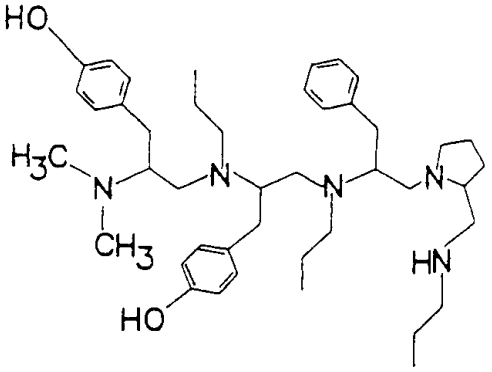

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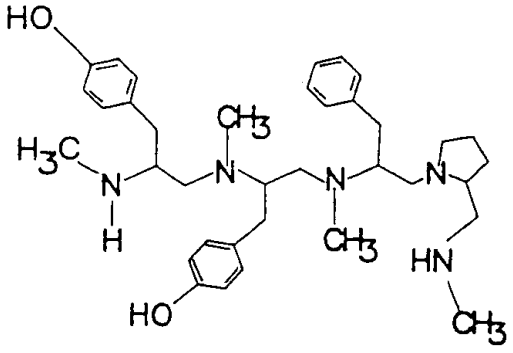

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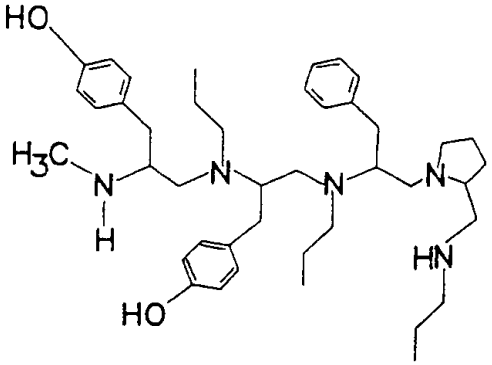

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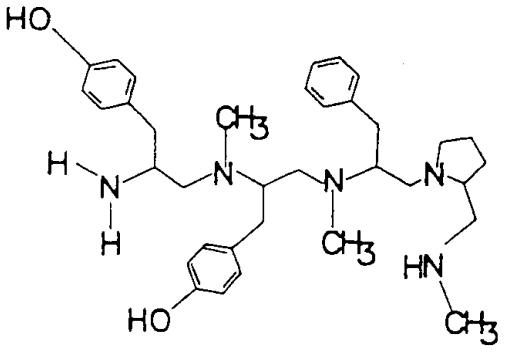

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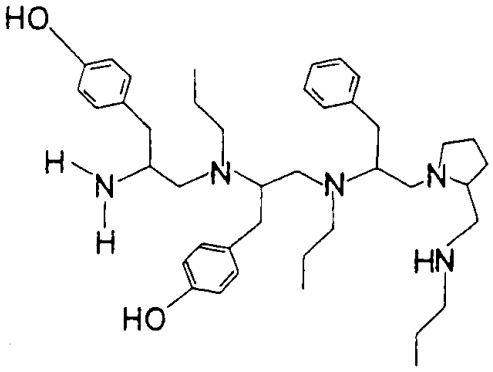

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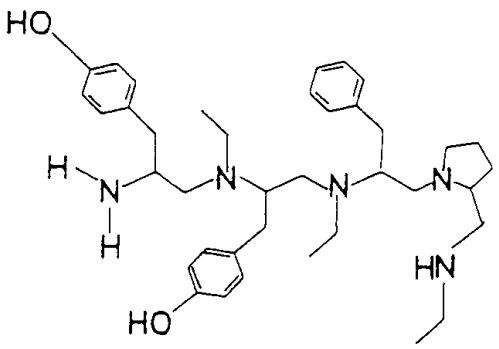
FIG. 4



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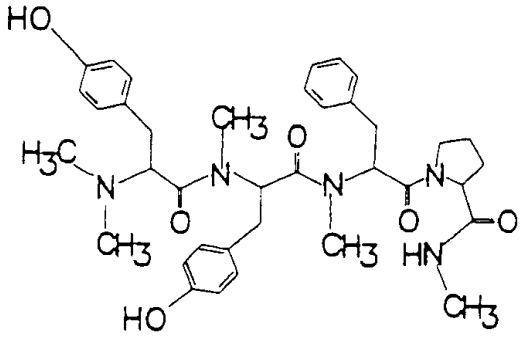

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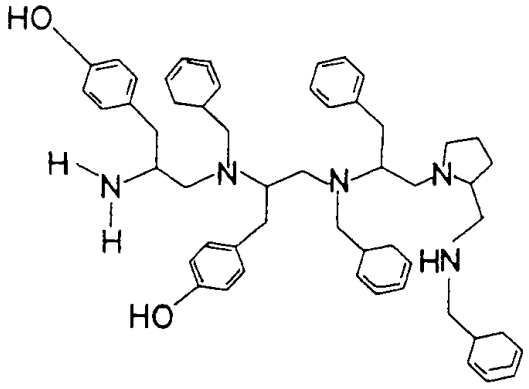

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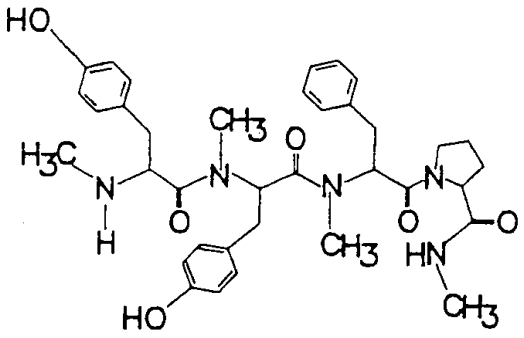

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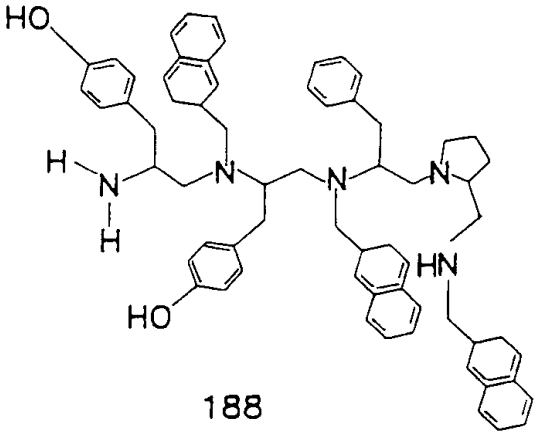

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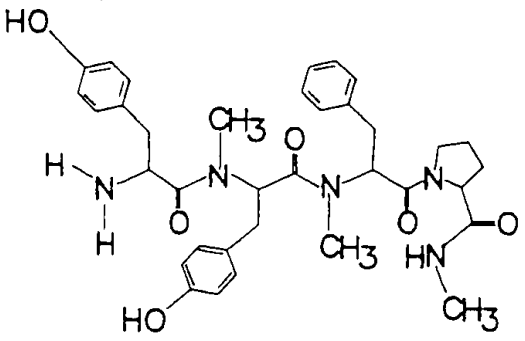

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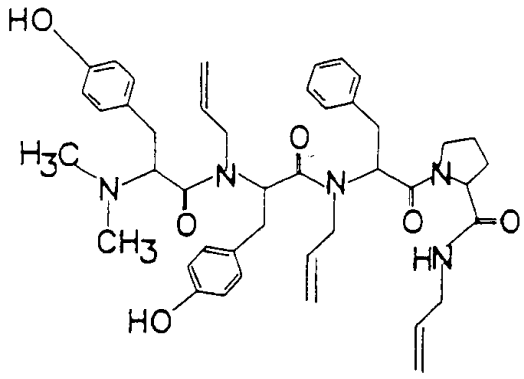
FIG. 5



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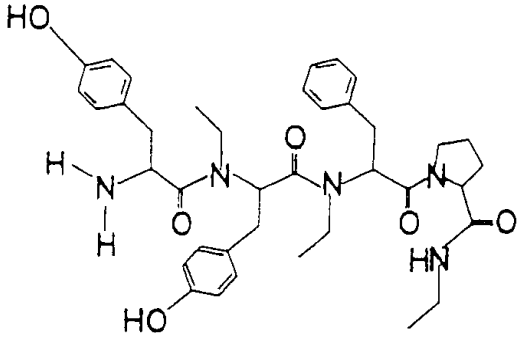

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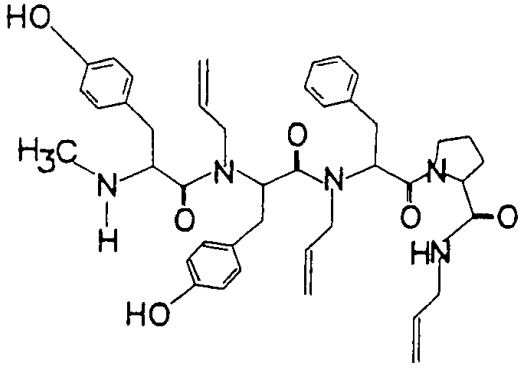

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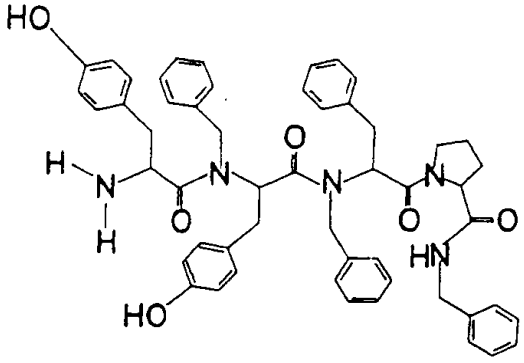

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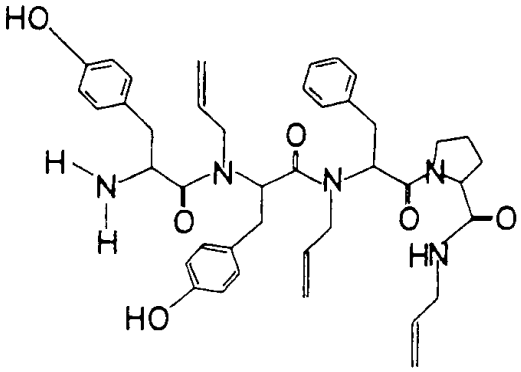

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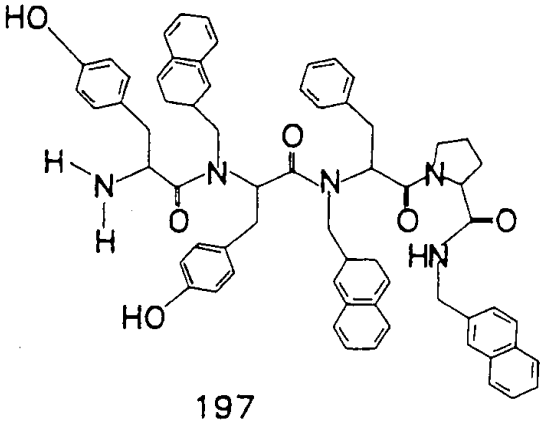

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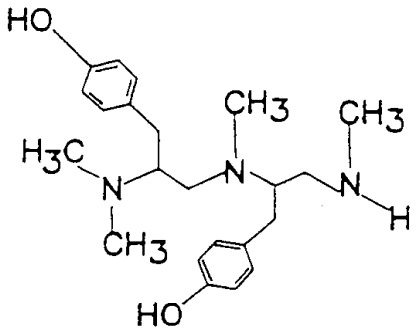
FIG. 6



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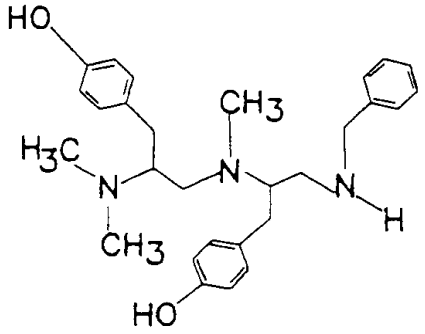

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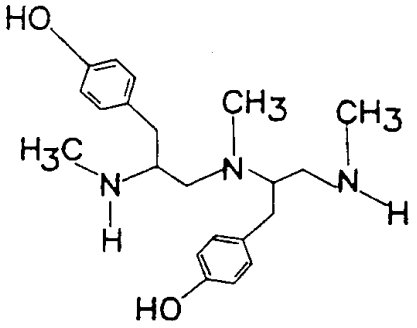

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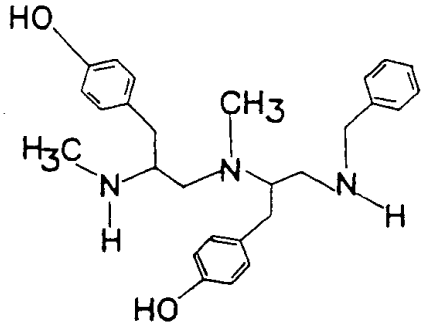

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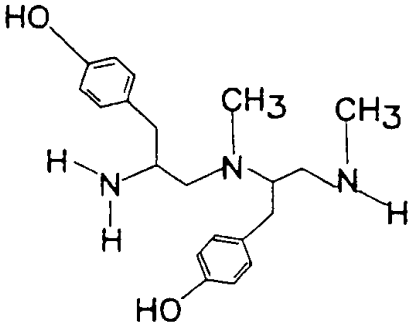

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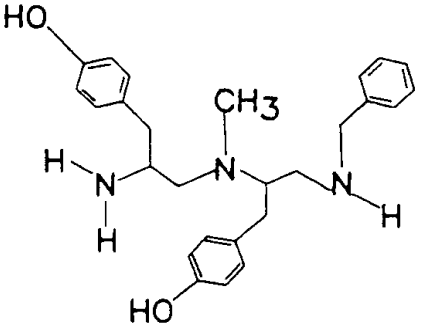

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FIG. 7